Supporting Information

Plastid Whirlies are Associated with Nucleoids

It has been previously shown that Whirlies of *Arabidopsis* localize to either plastids or mitochondria (1). An AtWhy1-green fluorescent protein (GFP) fusion produces punctuate foci of fluorescence in the plastids of transformed potato protoplasts, a pattern that is reminiscent of nucleoids (condensed DNA-protein complexes containing the complete plastid genome). Yet, co-localization of AtWhy1-GFP with plastid DNA has so far not been demonstrated. Therefore, we produced transgenic tobacco plants expressing a GFP fusion protein of StWhy1 (*Solanum tuberosum* Whirly 1), a close homolog of AtWhy1 (68% amino acid identity). Localization of StWhy1-GFP was monitored by laser-scanning confocal microscopy (LSCM). Examination of StWhy1-GFP fluorescence in transgenic leaf guard cells indicated that StWhy1 was also present in speckles within chloroplasts (Fig. S2A). To determine if ptWhirlies are associated with nucleoids, we transiently transfected protoplasts produced from tobacco mesophyll leaf cells with StWhy1-GFP. As shown in the upper right panel of Fig. S2B, StWhy1-GFP appeared as punctuate foci of fluorescence distributed in the chloroplasts. The transformed protoplasts were subsequently stained with the DNA-dye Syto85 which allowed the visualisation of nucleoids (lower left panel of Fig. S2B). Co-localization of Syto85 fluorescence with that of StWhy1-GFP in chloroplasts of tobacco mesophyll protoplasts (lower right panel of Fig. S2B) confirmed that StWhy1 co-localizes with DNA in plastids.

Plastidial Whirlies are Associated with ptDNA in a Non-Sequence Specific Manner

To characterize the DNA regions that are bound by AtWhy1 and AtWhy3, we performed DNA immunoprecipitation experiments followed by PCR detection of precise regions distributed all over the plastid genome : inside genes, around the determined transcription start sites of genes, in isolated regions that are devoid of any coding sequence and inside putative replication origins (Fig. S3A). No differences could be observed for the

immunoprecipitation of all types of regions between Col-0, KO1 and KO3 extracts, whereas no ptDNA could be immunoprecipitated using the KO1/3 extracts (Fig. S3B). To control for the specificity of interaction, we tested that the sonication regimen to which ptDNA was subjected yielded fragments of an average length of 500-1000 base pairs (Fig. S3C). This indicated that for any given region immunoprecipitated specifically, it can be assumed that there is at least one Whirly binding-site within a maximum of 1200 bp relative to the center of the amplified region. Additionally, we used primers to amplify a mitochondrial DNA region in the *atp9* gene as a negative control since AtWhy1 and AtWhy3 exclusively localize to chloroplasts and thus should not interact with the mitochondrial genome. We found that we could only amplify similar background levels of mitochondrial DNA for all genotypes, confirming the lack of specific interaction between AtWhy1/3 and mitochondrial DNA *in vivo* (Fig. S3D). These results indicate that ptWhirlies are able to bind many if not all ptDNA regions *in vivo*.

Supporting Information Materials and Methods

Arabidopsis mutant characterization

The SALK Institute Genomic Analysis Laboratory provided the sequence-indexed T-DNA insertion line SALK_099937 (2). The insertion inside the *AtWhy1* gene was mapped at nucleotide –102 relative to the initial ATG. The Seattle TILLING Project (3) provided plants with mutations in the *AtWhy3* gene. One line isolated and identified as il68D8 changed the TGG codon 138 to a TGA stop codon. The heterozygous M_2 plants were backcrossed with Col-0 eight consecutive times to eliminate background mutations. The homozygous progeny obtained was used for experiments. These *AtWhy3* mutant plants (KO3) were crossed with *AtWhy1* mutant plants (KO1) and the progeny was genotyped to isolate double mutants. The insertion mutant was genotyped using PCR primers specific for the wild-type or mutant allele. The KO3 point mutation was detected using PCR amplification with dCAPS primer W138for (CAGCTGGTGTTCGTCAATATGCCTG) coupled with primer W138rev

(GCCCAAGGCTAACTAGATTACCGAT) and digestion of the product with the MvaI restriction enzyme (Fermentas).

Microscopy

Chlorophyll autofluorescence in *Arabidopsis*

Mature leaf disks were taken from 4 week old plants and mounted on glass slides. A confocal microscope OLYMPUS IX71 was used with a 488 nm laser. Chlorophyll autofluorescence was visualised through a 660 nm long pass filter.

Transmission electron microscopy

Leaf mesophyll tissue from 4 week old plants was cut into 1-2 mm^2 pieces which were washed twice with 0.2 M sodium cacodylate buffer pH 7.4 for 10 minutes. Tissue was fixed by incubation for 6 hours in cacodylate buffer containing 1 % EM-grade glutaraldehyde. Samples were coated with epoxy resin, cut into 80 nm sections and mounted onto nickel/formvar grids. Staining of the samples was done using uranyl acetate and lead citrate. Observations were done using a JEOL (JEM1230) transmission electron microscope at 80 kV. Photographs were taken using a Gatan DualVision camera.

Intracellular localization of StWhy1

The StWhy1-GFP construct was produced by inserting the Emerald GFP (Clontech) sequence downstream of *StWhy1* into pBluescript (Stratagene). The StWhy1-GFP fusion comprising all 274 amino acids of StWhy1 was then inserted into the pBin19 vector which contains 2 copies of the CaMV35S promoter. All constructs were electroporated into *Agrobacterium tumefaciens* strain LBA4404 and transgenic plants were obtained as described (4). For transient expression, the StWhy1-GFP fusion in pBin19 was transferred to the pBI223 vector which also contains the CaMV35S promoter. Leaf mesophyll protoplasts were isolated and transformed as described. GFP fluorescence (500-530 nm) was visualised with a Leica DM IRB/E laser-scanning confocal microscope using a 488 nm laser excitation source. Chlorophyll was excited using a 543 nm laser and its autofluorescence was visualised at 575-630 nm. For DNA staining, protoplasts were incubated with 5 μM Syto85 (Molecular Probes) for 10-30 min and then

washed with fresh culture medium. Syto85 fluorescence (570-600 nm) was visualised using a 568 nm laser excitation source. GFP and Syto85 images were collected sequentially, and no fluorescence cross-talk was observed under our conditions (data not shown). Pseudocoloring of the images, maximal projections, and image overlays were done using the Leica confocal software. Hand-made thin sections of fresh leaves from 4 weeks-old plants were examined in sterile water. Confocal microscopy was carried out using an Olympus FV300 microscope.

Plastid DNA immunoprecipitation

Leaf tissue was fixed in 1 % formaldehyde for 15 minutes under vacuum. Glycine was added to 0.125 M to titrate the remaining formaldehyde and fixed tissue was washed 3 times with distilled water. Crude plastids were prepared by grinding in chloroplast extraction buffer (0.33M sucrose, 10 mM MES-NaOH pH 4.6, 10 mM NaCl, 1 mM EDTA, 10 mM DTT) using a mortar and pestle. The ground tissue was filtered through 2 layers of Miracloth (Calbiochem) and spun at 1000 g for 5 minutes in a microcentrifuge to pellet most plastids. The pellet was resuspended in IPP buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 1 % NP-40, 0.5 % sodium deoxycholate and 1 tablet of Complete Protease Inhibitor (Roche)), lysis was carried out on ice for 2 minutes and the lysate was spun at 11000 g for 15 minutes. Supernatant was sonicated using a Branson microtip sonicator. Sonicated material was precleared by incubating with protein-A-agarose beads (Roche) for 1 hour at 4ºC on a rotating platform. The precleared material was spun at 11000 g for 15 minutes and the supernatant was transferred to a fresh tube. Proteins were quantified and an 100 µg aliquot was kept as input material while 500 µg was used for immunoprecipitation using 1/200 rabbit polyclonal anti-AtWhy1 sera in 1 mL final volume. Immunoprecipitations were carried out for 1 hour at 4ºC before adding 50 µl of protein-A-agarose beads. Complexes were allowed to form overnight. Beads were pelleted at 100 g for 1 minute and were washed 2 times 5 minutes with IPP buffer, 2 times 5 minutes with low salt buffer (50 mM Tris-HCl pH 7.5, 0.1 % NP-40, 0.05% sodium deoxycholate), 2 times 5 minutes with high salt buffer (50 mM Tris-HCl pH 7.5, 500 mM NaCl, 0.1 % NP-40, 0.05% sodium deoxycholate), 2 times 5 minutes with LiCl

buffer (50 mM Tris-HCl pH 7.5, 0.1 % NP-40, 0.05 % sodium deoxycholate, 250 mM LiCl) and 2 times 5 minutes with TE pH 7.5 (10 mM Tris-HCl pH 7.5, 1 mM EDTA). Beads were pelleted at 11000 g for 20 seconds and resuspended in TE buffer containing 1 % SDS. Complexes were eluted from the beads by heating at 65ºC for 15 minutes. Beads were spun down at 11000 g for 5 minutes and the supernatant transferred to a new tube. One tenth volume of 2 M NaCl was added and the eluted complexes were incubated at 65ºC overnight for de-crosslinking. Glycogen was added to a final concentration of 0.5 µg/µl and two volumes of 100 % ethanol was added. DNA was precipitated at –80ºC for 1 hour. Tubes were spun at 11000 g for 20 minutes and DNA pellets were resuspended in TE buffer. DNA was subjected to a phenol/chloroform extraction and to a chloroform extraction before being reprecipitated as above. After a final wash with 70% ethanol, DNA was resuspended in water. This DNA was used for PCR reactions. To further control the specificity of the experiment, we determined the size of DNA fragments following sonication and found that they were between 500 and 1200 bp in length. For the determination of the specificity of the interaction between AtWhy1 and AtWhy3 and plastid DNA, mitochondrial DNA immunoprecipitation was performed as described previously (5).

Electrophoretic mobility shift assays

Five hundred mg of leaves taken from 4 week old plants were ground in 5 mL of ice-cold chloroplast isolation buffer (0.33 M sucrose, 10 mM MES-NaOH pH 4.6, 10 mM NaCl, 1 mM EDTA, 10 mM DTT). The resulting slurry was filtered through 2 layers of Miracloth (Calbiochem). The suspension was then centrifuged at 3000 rpm in a microcentrifuge for 5 minutes. The crude plastids were resuspended in IPP buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 1 % NP-40, 0.5 % sodium deoxycholate, 1X Complete protease inhibitor cocktail (Roche)) and incubated 2 minutes on ice. The lysed plastids were centrifuged at maximal speed in a microcentrifuge for 15 min and the supernatant was taken. Twenty µg of the crude plastid extracts were used for ssDNA binding assays. The same extracts were used in the protein gel blot presented Figure 1B thereby providing a loading control. For electrophoretic mobility shift assays, crude plastid proteins were incubated in IPP buffer

with 30000 counts per minute of end-labelled modified 4XPB (6) oligonucleotide (5'-TGTCATTTTTGTCATTTTTGTCATTTTTGTCA-3') for 30 minutes at room temperature. The anti-AtWhy1/3 antibody or preimmune serum were added to the Col-0 extracts before adding the oligonucleotide. The reactions were then separated on a 5.4% polyacrylamide Tris-Borate-EDTA gel as described (6).

Isolation of RNA

RNA was isolated from 4 week old plants using TRIZOL® reagent (Invitrogen) according to manufacturer's instructions. Running of the samples was performed as described (7). For the Var A and Var B samples, only highly variegated tissue was used.

Detection of rearranged DNA by PCR in maize

PCR reactions were conducted using the Ex Taq polymerase from Takara Bio Inc. according to the manufacturer's instructions. The search for rearranged products was performed using a series of outward-facing oligonucleotides spaced by approximately 5-50 kb. A total of 19 PCR reactions were performed on each of two independent DNA samples from B73 inbred line (WT) and each of the *ZmWhy1* mutant lines and analysed by gel electrophoresis. The DNA samples for all the plants were pre-adjusted to the same amount of ptDNA using semi-quantitative amplification of the maize *rpoB* gene sequence before performing the outward-facing PCR. All visible DNA bands were isolated, cloned and sequenced.

Oligonucleotides used in this study

The following oligonucleotides were used to identify rearranged products in *Arabidopsis*:

5291REV, TCC CAA CGA GCC GTT TAT CGA ; 9121REV, AAG ATA GAT CGG GCC AAC TCG ; 9640FOR, GGT TCA AAT CCT ATT GGA CGC; 19181FOR, CAG CAT CTG ATG TGC GTA CAG ; 19309FOR, TCG TCT GCT AAG ACA CGA CCA ; 20481, FOR GCG TTG ATC TAA TTG CCA CCG ; 20481REV, CGG TGG CAA TTA GAT CAA CGC ; 30511FOR, GCT GCC TCC TTG AAA GAG AGA ; 30511REV, TCT CTC TTT CAA GGA GGC AGC ; 31138REV, AAT AGC CTA GCG CAT CGA ACG ; 40130FOR, TGA CAT GTT CCT CCT CTT CCC ; 40130REV, GGG AAG AGG AGG AAC ATG TCA ; 48481FOR, AGG GCA AAA ATA CCC CAA GGT ; 50251FOR,

GGC CCC TTC CTA ATT GGA GAT ; 50513FOR, GGT TGT TGT GGT AGA CGT CTC ; 50513REV, GAG ACG TCT ACC ACA ACA ACC ; 54551REV, TGG GAC GCA TAA CCG GAT ATG ; 58730FOR, CCT CCC TTG CTT GCA TAT GTA; 59291FOR, AGG CCT AGT CTT TCC GGC AAT ; 59291REV, ATT GCC GGA AAG ACT AGG CCT ; 60182REV, ATA TAA AGG ACA CGC CGG GCA ; 65482REV, GCA TCT GCA CTA TCT CAG GAG ; 68821FOR, CTC CGA GCT ATA TAT CCG CGT; 68821REV, ACG CGG ATA TAT AGC TCG GAG; 69633FOR, ACA CCC GAG TAC ATG TTC CTC; 69633REV, GAG GAA CAT GTA CTC GGG TGT; 69941REV, AGC AAC AGA AGC CCA AGC TCA; 71221REV, TTC TTG TTC CTG AAC GGG CTT ; 79920FOR, CCA TGC CGT TCG GAA GTG ATT ; 79920REV, AAT CAC TTC CGA ACG GCA TGG ; 84761FOR, TAT GTA TGG CCG TGC CTA AGG ; 84761REV, CCT TAG GCA CGG CCA TAC ATA ; 95260FOR, AAC CTG CTA GTG GAG GAA GAC; 95260REV, GTC TTC CTC CAC TAG CAG GTT ; 104831FOR, CTG AAT CCA TGG GCA GGC AAG ; 105983REV, GCG TTA CTC AAG CCG ACA TTC ; 111330FOR, TGC GGT TAA TCC CGC TGT TGA ; 111330REV, TCA ACA GCG GGA TTA ACC GCA ; 115987FOR, AAA CCA CTC ATG CCC GGC AAT ; 116007FOR, AAA CCA CTC ATG CCC GGC AAT ; 116007REV, ATT GCC GGG CAT GAG TGG TTT ; 121141FOR, AAC GTC ATC CGG TTA CCG GTT.

The following oligonucleotides were used to identify rearranged products in maize:

Zm33FOR, GGG TAT TTC CGG CTT TCC TTC ; Zm33REV, GAA GGA AAG CCG GAA ATA CCC ; Zm4761FOR, TCT CCT CAT ACG GCT CGA GAA ; Zm4761REV, TTC TCG AGC CGT ATG AGG AGA ; Zm10113REV, TTC CAC GTG GTA GAA CCT CCT ; Zm15001REV, TAC CCC TTG GAA ATG GGG TAC ; Zm20058FOR, CCA ACT TAG GGG GGT ACG AAT ; Zm20058REV, ATT CGT ACC CCC CTA AGT TGG ; Zm25060FOR, GCA TGT CCA GTG ACT CAT GTG ; Zm25060REV, CAC ATG AGT CAC TGG ACA TGC ; Zm29971REV, AAC AAG AGA AGC ACG AGC TCC, Zm35230FOR, GAA AGG GAG TGT GTG CGA GTT ; Zm40020FOR, CCC AAG GTA TGG AAT CCT AGG ; Zm40020REV, CCT AGG ATT CCA TAC CTT GGG ; Zm45152FOR, TTT CGT TCT AGC GCC CGG AAA; Zm45152REV, TTT CCG GGC GCT AGA ACG AAA ; Zm50141FOR, AGA GGT GAG ATT AGG TGC CCT ; Zm50141REV, AGG GCA CCT AAT CTC ACC TCT ; Zm54971FOR, TGC CAA CAA TCC GAG GCT GTA ; Zm54971REV, TAC AGC CTC GGA TTG TTG GCA; Zm59880FOR, CGG GCC TAT TCA TTA GCT CCT ; Zm59880REV, AGG AGC TAA TGA ATA GGC CCG ; Zm64883FOR, GGG GTC GTG GAA TGC TTT TCT ; Zm64883REV, AGA AAA GCA TTC CAC GAC CCC ; Zm69981FOR, ATT AGC CAA CCG CCA AGG GAA ; Zm69981REV, TTC CCT TGG CGG TTG GCT AAT ; Zm74851FOR, GCG TAA GTC CCC TAT CTA GGA ; Zm85072FOR, ACC TCT CCG GAT CCT CGA ATT ; Zm89967FOR, GAA AAG CAA CGA CTG GAG TGG ;

Zm89967REV, CCA CTC CAG TCG TTG CTT TTC ; Zm100051REV, TCG ACG ATG AAG CTT ATC CCC ; Zm105133FOR, ATT CGC CCT CCC CCT ACA TAT ; Zm105133REV, ATA TGT AGG GGG AGG GCG AAT ; Zm110101FOR, CTG GAA TAT AGA GCG GAC TGG ; Zm129981FOR, GAG CAG GCT ACC ATG AGA CAA ; Zm129981REV, TTG TCT CAT GGT AGC CTG CTC ; Zm135011FOR, TCA GTG ACT TTG GCA CTG GAC.

The following oligonucleotides were used to generate probes for DNA gel blots in *Arabidopsis*:

421FOR, AGC AGC TAG GTC TAG AGG GAA ; 5291REV, TCC CAA CGA GCC GTT TAT CGA ; 10230FOR, GCA ATC GTT GAC CTC TTG CCA ; 15461REV, CAA TAC GGG CTC GGT GTC ATT ; 30511FOR, GCT GCC TCC TTG AAA GAG AGA ; 35850REV, ACC AAC CAT CAG GAG ACG CAA ; 40130FOR, TGA CAT GTT CCT CCT CTT CCC ; 44916REV, GTA CGG AAA GAG AGG GAT TCG ; 49741FOR, CCT TAC GTA AAG GCC ACC CTA ; 54551REV, TGG GAC GCA TAA CCG GAT ATG ; 54551FOR, CAT ATC CGG TTA TGC GTC CCA ; 59291REV, ATT GCC GGA AAG ACT AGG CCT ; 59291FOR, AGG CCT AGT CTT TCC GGC AAT ; 64485REV, CGT TGC TGT GTC AGA AGA AGG ; 69633FOR, ACA CCC GAG TAC ATG TTC CTC ; 74901FOR, CAG AGA ATG GGG TCT GTC ATC ; 79920REV, AAT CAC TTC CGA ACG GCA TGG ; 79920FOR, CCA TGC CGT TCG GAA GTG ATT ; 84761REV, CCT TAG GCA CGG CCA TAC ATA ; 84761FOR, TAT GTA TGG CCG TGC CTA AG ; 90150REV, TCC ATC TGC CCT TTG TCA ACG ; 100770FOR, GAA GTC ATC AGT TCG AGC CTG ; 105983REV, GCG TTA CTC AAG CCG ACA TTC ; 105983FOR, GAA TGT CGG CTT GAG TAA CGC ; 111330REV, TCA ACA GCG GGA TTA ACC GCA ; 111330FOR, TGC GGT TAA TCC CGC TGT TGA ; 116007REV, ATT GCC GGG CAT GAG TGG TTT.

The following oligonucleotides were used to determine the ends of amplified regions in *Arabidopsis* variegated lines:

56061REV, AGA ACA CCA GGT AGT GAG ACC ; 60211FOR, GAG GTC AAG GGG CTA TTC CTT ; 63990REV, CAT GGA CTA GCT GTA CCT ACC ; 81360FOR, CGC AGG TCT TAC TGT AAC TGG ; 10230REV, TGG CAA GAG GTC AAC GAT TGC ; 30511FOR, GCT GCC TCC TTG AAA GAG AGA ; 62161REV, TAG CAG GGT CTG GAG CAA GAA ; 75961FOR, TTA CTG GTG TGG TTC TGG GTG ; 120601FOR, GCG CTT CGG AAT TCA TCT CAC ; 4341FOR, CCG AGT ACT CTA CCG TTG AGT.

The following oligonucleotides were used for adjusting DNA levels in the PCR experiments:

YCF2FOR, GAT CTC TGA GAG CTG TTT CCG ; YCF2REV, TGT TTC GCC TCT TAC TCG GAG ; ZmRpoBFOR, GCT TGG CGG AAG AAC TTG AGA ; ZmRpoBREV, TGT TTC GCC TCT TAC TCG GAG.

The following oligonucleotides were used to generate probes for DNA gel blots in maize:

Zm4761FOR, TCT CCT CAT ACG GCT CGA GAA ; Zm10113REV, TTC CAC GTG GTA GAA CCT CCT ; Zm10113FOR, AGG AGG TTC TAC CAC GTG GAA ; Zm15001REV, TAC CCC TTG GAA ATG GGG TAC ; Zm15001FOR, GTA CCC CAT TTC CAA GGG GTA ; Zm20058REV, ATT CGT ACC CCC CTA AGT TGG ; Zm25060FOR, GCA TGT CCA GTG ACT CAT GTG ; Zm29971REV, AAC AAG AGA AGC ACG AGC TCC ; Zm29971FOR, GGA GCT CGT GCT TCT CTT GTT ; Zm35230REV, AAC TCG CAC ACA CTC CCT TTC ; Zm35230FOR, GAA AGG GAG TGT GTG CGA GTT ; Zm40020REV, CCT AGG ATT CCA TAC CTT GGG ; Zm40020FOR, CCC AAG GTA TGG AAT CCT AGG ; Zm45152REV, TTT CCG GGC GCT AGA ACG AAA ; Zm45152FOR, TTT CGT TCT AGC GCC CGG AAA ; Zm50141REV, AGG GCA CCT AAT CTC ACC TCT ; Zm50141FOR, AGA GGT GAG ATT AGG TGC CCT ; Zm54971REV, TAC AGC CTC GGA TTG TTG GCA ; Zm59880FOR, CGG GCC TAT TCA TTA GCT CCT ; Zm64883REV, AGA AAA GCA TTC CAC GAC CCC ; Zm69981FOR, ATT AGC CAA CCG CCA AGG GAA ; Zm74851REV, TCC TAG ATA GGG GAC TTA CGC ; Zm74851FOR, GCG TAA GTC CCC TAT CTA GGA ; Zm79860REV, TGA AGG GAA AAT CCT GCC GAG ; Zm85072FOR, ACC TCT CCG GAT CCT CGA ATT ; Zm89967REV, CCA CTC CAG TCG TTG CTT TTC ; Zm95042FOR, GTG GCG AAC TCC AGG CTA ATA ; Zm100051REV, TCG ACG ATG AAG CTT ATC CCC ; Zm100051FOR, GGG GAT AAG CTT CAT CGT CGA ; Zm105133REV, ATA TGT AGG GGG AGG GCG AAT ; Zm105133FOR, ATT CGC CCT CCC CCT ACA TAT ; Zm110101REV, CCA GTC CGC TCT ATA TTC CAG ; ZmMt255542FOR, GCA GTA TTG GAA CCA TCC TCG ; ZmMt259720REV, CCC ATG ATG TGG TAA AGG GAG.

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